## AMENDMENTS TO THE SPECIFICATION

Please add a cross-reference section on page 1 after the title to read as follows:

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a continuation of Application No. 09/531,050, filed March 20, 2000, which is a continuation of Application No. 09/098,082, filed June 16, 1998, now U.S. Patent No. 6,040,421, which is a divisional of Application No. 08/765,081, filed March 26, 1997, now U.S. Patent No. 5,798,260, which is the national stage of International Application No. PCT/US95/06994, filed June 7, 1995, which is a continuation-in-part of Application No. 08/265,714, filed June 24, 1994, now abandoned.

Please amend the paragraph beginning on page 4, line 11, to read as follows:

Transposon-mediated mutations of *E. coli* O157:H7 have been isolated that do not adhere to HeLa cells and that have lost the ability to colonize bovine intestines. A HeLa cell *in vitro* system has been established that provides a means of assaying variants of *E. coli* 0157:H7 for their ability to colonize cattle. The gene into which the transposon inserted have been sequenced. In a ~~seperate~~ separate approach, two overlapping 40 kb segments of chromosomal DNA from *E. coli* O157:H7 have been cloned that confer D-mannose resistant adherence to nonadherent strains of *E. coli*. The overlapping region has been cloned, and *E. coli* HB101 expressing this overlapping region on a plasmid (ATCC No. 69648) have acquired the ability to adhere to epithelial cells of both human (HeLa) and bovine (MDBK) origin. These findings demonstrate that a contiguous segment of chromosomal DNA from *E. coli* O157:H7 encodes an adhesin, and that this same adhesin mediates both bacterial adherence to HeLa cells.

Please amend the paragraph beginning on page 4, line 22, to read as follows:

The adhesin-encoding region of p*ear* has been identified as the nucleotide sequence of SEQ ID NO:4. Also described are recombinant expression vectors containing the adhesin-

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

coding region, as are bacterial cells which are transformed with the recombinant expression vector. The recombinant adhesin preferably has the amino acid sequence of SEQ ID [[NO: 4]] NO:5. Also described are immunological binding partners that bind to the recombinant adhesin. Vaccine formulations of the invention contain the recombinant adhesin encoded by a nucleic acid molecule that hybridizes under stringent conditions to the nucleotide sequence of SEQ ID NO:4.

Please amend the paragraph beginning on page 8, line 23, to read as follows:

The appended SEQ ID NO:1 shows the 8,041 base pair nucleotide sequence of p*ear*. Almost all of the sequence has been confirmed. Ambiguous DNA (in regions not encoding the candidate adhesin) is noted by N in the appended sequence. The p*ear* insert contained three open reading frames (ORFs) of sufficient length to encode potential virulence or adherence factors. Two of these are homologous to genes necessary for resistance to tellurite (Jobling, MG, et al., Gene 66:245-258, 1988). These *terE* and *terD* homologs are shown in SEQ ID NO:2 and SEQ ID NO:3, corresponding respectively to nucleotides 7024-6449 and 7670-7092 of SEQ ID NO:1. The other ORF is homologous to a gene encoding a homolog of IrgA (Goldberg, MB, et al., Molecular Microbiology 6:2407-2418, 1992). This *irgA* homolog is shown in SEQ ID NO:4, which corresponds to nucleotides 3036-5126 of SEQ ID NO:1. IrgA is an outer membrane protein of *V. cholerae*, and is believed to be important for colonization of mice in an experimental system (Goldberg, MB, et al, Infection and Immunity, 58:55-60, 1990). The *E. coli* O157:H7 adhesin (SEQ ID [[NO: 4]] NO:5) is also homologous to the *E. coli* colicin I receptor (CIR) (Griggs, D.W., et al., J. Bacteriol. 168:5343-5352, 1987). The amino acid homologies of the candidate adhesin to IrgA and to CIR are demonstrated by comparing SEQ ID NO:5 and SEQ ID NO:6, and SEQ ID NO:7 ~~and SEQ ID NO:8~~, respectively.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

Please amend the paragraph beginning on page 9, line 12, to read as follows:

A product of a single gene (i.e., the *irgA* homolog) confers adherence to nonadherent *E. coli*. We first performed PCR using as primers the sequences 5'GGGGATCCAATTCTGGCATGCCGAGGCAGTCG3' (SEQ ID [[NO:9]] NO:8), corresponding to nucleotides 2895-2914 of SEQ ID NO:1) and 3'GGACCGCCTTGTCACCGTTGCTCTTAGATCTGG5' (SEQ ID [[NO:10]] NO:9, corresponding to nucleotides 5176-5196 of SEQ ID NO:1) from which DNA on *pear* was amplified. These sequences were cloned into the *Bam*HI and *Xba*I sites of pSK+. We also amplified the same gene using as template DNA from *E. coli* O157:H7. In this latter case,. the primers used were 5'GGAAGGATCCCCGAACACGCCATACGGATAGCTG3' (SEQ ID [[NO:11]] NO:10, corresponding to nucleotides 2867-2890 of SEQ ID NO:1) and 3'GCAACGGTGACGTTGAGGACCGCCAGATCTAAAGG5' (SEQ ID [[NO:12]] NO:11, corresponding to nucleotides 5159-5183 of SEQ ID NO:1). This latter PCR product was also cloned into pSK+, using the same *Bam*HI and *Xba*I sites. In both cases, multiple laboratory strains of nonadherent *E. coli* were rendered adherent to HeLa cells by these cloned single genes.

Please amend the paragraph beginning on page 10, line 18, to read as follows:

We have also performed Tn*phoA* mutagenesis of *E. coli* O157:H7, and identified three nonadherent mutants (strains A5, F4, and N11), each of which sustained a Tn*phoA* insertion in the same allele (SEQ ID [[NO:9]] NO:12). One of these strains, strain F4, was deficient in its ability to colonize in calves in an oral challenge experiment performed at the Washington State University in Pullman, Washington. Sequence analysis suggests that the Tn*phoA* insertion in the same allele among the three nonadherent mutants may have taken place in the midst of a cluster of genes, at least one of which has homology to pro-secretory proteins in *Yersinia enterocolitica* (YscJ) (Michiels, T., et al., Journal of Bacteriology 173:4994-5009, 1991), *Rhizobium fredii*

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

(Nolt) (Meinhardt, L.W., et al., Molecular Microbiology 6:2407-2418, 1992), and *Xanthomonas compestris* (HrpB) (Fenselan, S., et al., Molecular Plant-Microbe Interactions 5:390-396, 1992), and it is possible that the secretion of the *E. coli* O157:H7 adhesin is controlled by this secretory mechanism.

Please amend the paragraph beginning on page 12, line 34, to read as follows:

Standard immunochemical techniques are used to determine if the cloned adhesin (SEQ ID [[NO:4]] NO:5) is the same as the adhesin used by *E. coli* 0157:H7 to adhere to HeLa cells. To achieve this objective, outer membrane proteins are prepared from laboratory strain(s) expressing the recombinant adhesin (SEQ ID [[NO:4]] NO:5). These proteins are analyzed on SDS-polyacrylamide gels, and used to immunize rabbits at three one-month intervals and Holstein cows at 30 and 60 days prepartum. Rabbits are also [[be]] immunized with killed whole bacterial cell preparations. A recombinant *E. coli* NM554, which does not adhere to HeLa cells, is used as a negative control immunogen.

Please amend the paragraph beginning on page 14, line 32, to read as follows:

Types Of Vaccines: Purified antigen (prepared using standard recombinant DNA methods) or whole-cell vaccines can be used to stimulate an immune response in pregnant cows, resulting in the presence of protective antibodies in the ~~colustrum~~ colostrum and milk produced after parturition. The milk or ~~colustrum~~ colostrum can be stored for later administration to newborn calves. Alternatively, protein produced from the *irgA* homolog can be used to raise monoclonal antibodies which then can be used directly to confer passive immunity to newborn calves. Methods for preparing monoclonal antibodies are well-known in the art, and can be found, for example, in Harlow and Lane, *Antibodies*, Cold Spring Harbor Laboratory, 1988.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

Please amend the paragraph beginning on page 16, line 9, to read as follows:

Injection: Four to eight ml doses of the vaccine are administered intramuscularly or directly into the lactiferous ducts via the teat canals of two year old cows. For lactiferous duct administration, the cows are given a series of 4 to 6 ml doses at 42, 32, 22 and 12 days prior to parturition. For intramuscular vaccination, 4 ml of the formalinized culture is injected in the side of the neck 28 days before parturition, followed by 6 ml 14 days later, and 8 ml at 5 days before the expected calving day. About 1 liter of milk is collected from vaccinated and control cows seven days after parturition. Whey is prepared by centrifuging milk [[for]] at 44,000 g for two hours, then collecting the supernatant. Whey is stored either in 2 ml aliquots at 4°C, stored frozen at -30°C, or stored lyophilized.

Please amend the paragraph beginning on page 20, line 2, to read as follows:

pSC(overlap): deletion mutant of pSC(A-G6) which retains 15 kb overlap region, and confers adherence to *E. coli* HB101. pSC(overlap) was deposited on June 24, 1994, under accession number 69648 at the American Type Culture Collection, ~~12301 Parklawn Drive, Rockville, MD 20852,~~ 10801 University Boulevard, Manassas, VA 20110-2209, U.S.A.

An Abstract of the Disclosure has been added at the end of the specification as a new page.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100